

Transformation control

January 9-10, 1947.

P9. broz YB - Y53, ~~Y57~~. Y40

10A10. broz ($\frac{100}{50}$ ml NSB) Y53, Y40 (A)

1P10 broz YB - Y40 (B)

4-5P10. Wash (A) cells.

1. Mix Y53-Y40 cells. ✓

8P10.

Suspend Y53 (A) cells in T-minimal. incubate 3 shaking 3h.

Sediment (C, D) and mix with washed Y40 (B). 2. plate 0.

3. ~~Mix~~ supernatant of C, D + mix \bar{c} washed Y40 (B) 3. plate 0.

1.5×10^2 prototrophs.

2. C $> 10^2$ " turbid for count.
D No?

3. filtrate:

C - 1 prototroph ?? } supernatant was not entirely free of cells
1 ?? } by the centrifugation. Repeat \bar{c} controls

on influence of dilution of 1 cell type on prototroph yield.

Recombination types

January 10, 1947.

Y40 + Y53 in T(0), T(B₁) agar

Pick colonies to EMB lactose. 1/12/47 ~~1-15~~ 7-15. 8, 13 +
others -

Streak out densely on (A) BMTL-lactose (B) BMTL lactose + glucose.

Compare the B₁⁻ types & types indistinguishable from these plates.

Colony-Plate:	0 = B ₁ ⁻ only	A	B	C	D	E	F	G
lac 1	-S ^(+R)	-S	-S	-S	-S			
lac 2	-							
* lac 3	-R	+S ^(+R)	-S ^(+R)					
* lac 4	-S	+S ^(+R)	-S ^(+R)	±S ^(+R)	±S ^(+R)			
lac 5	-S ^(±R)	-S	-S	+R ^(-R)				
lac 6	-R ^(±R)	-R	-R	-R	-R	-R		
6 8	* -S	-S	-S	-S	-S	-S	-S	+R
6 11	-R	-R	-R	-R	-R	-R	-R	-R
6 12	-S	-S	-S	-S	-S	-S		all mixed -R
6 13	+R	+R	+R	+R	+R	+R	+R	
* 6 15	-R	-S	-S	-S	-S			

BM +R
TLB, -S



Segregation of T_1^R

386

January 11, 1947.

Y64 x 58-161.

TLB, lac- T_1^R x BM lac+ T_1^S

good material.

a. prototrophs

T_1^R lac- R	+	S	-	S	+
42	1	53	23		

R = 36% 64%

lac- = 80%

b. B₁ plates . Much more numerous colonies (10x)

(not well readable).
(colonies impure!).

8	0	5	10
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Segregation of theoid.

January 11, 1947

a. Y67 (Y53M) x 58-161 (Tay x Y40.)

shenon lac+

Muc lac - Sm lac - Muc lac + Sm lac +

P) 17 P) 9

Lac- = .66

~~Y57 (Y53/3,15, M) x 58-161 (Tay x Y40)~~

E Y68 (58-161M) x Y53. (Tay x Y64)

segregation: ~~ML- ML+ ml- ml+~~

6 P 22 P 12 7

M₆₈ linked to lac⁻

Interaction of
expression of
Lac- + Muc+
on EMBlac
medium?

note variation in
shen. kinetics
character?

January 11, 1946.

P10 has 100 ml (125 fl. YB - Y53.)

N11. Centrifuge 250 ml (step 25-1). Suspend cells in

15 ml .9% NaCl. Add benzoyl + incubate for autolysis or
shaken at 25°. (12:45 PM - 3:20 PM.) Centrifuge "free cells" and

Mix 5 ml \bar{c} 1 ml Y40 suspension + plate 3 x 2 ml samples
into T(0) agar.

P14. colonies:

ca 10 large $10^{2.5}$ small \bar{c} colonies. v. clear plates. sign?

See 394

January 11, 1948.

Plate Y55 (+... *lac*-) into lactose - minimal at various dilutions: (Ass. = 10^9)

10^8 est. cells
Discretely crowded.

10^6 about 10^4 visible colonies

10^4 about 200 large colonies, with halos of small ones. Small cols. much smaller than below.

10^2 about 5x. 10^3 small colonies; 6 typical colonies (probably *lac*+). Difficult

1) The reversion frequency, as estimated from EMB plates is very high (ca 10^{-4} to 10^{-3} /generation??)

2) At least on this medium, *lac*- is capable of developing to some extent.

Since they develop halos, it is likely that there is a limiting factor in the agar which faintly permits growth.

Test large colonies on EMB:

Segregation of T_2^R

390

Jan 11, 47

465 x 58-161

No colonies!!

(Repeat!)
not turbid - prob mix error.

Repeat Jan 15.

loaded!

High rate????

January 11-12, 1947.

P 11 Inoz YB - Y43, Y44.

230P12 - Inoz YB = Y43, Y44, Y43+Y44, Y43+Y53.

Y P12. Wash + plate.

(Y43) + (Y44)	0, 0	red furbid (acc.)
(Y43+Y44)	0	
(Y43+Y53)	0	
(Y43)+(Y53)	0	

Storage + recombination

January 12, 1947

Plate Y40 + Y53 (cultures as in 391) in T(0) as initial controls.

9-10 P12. .5 ml. 25

T.O. - ca 2-300.

a. Keep Y40, Y53 in water (.9% NaCl) 25°. Mix P13.

b. Keep (Y40 + Y53) in water. Plate 4 P13

c. Keep Y40, Y53 in T(0). [Add 1 ml to 10 ml T(0)]. Mix + plate 4 P13

[d.] P12. Plate Y40, Y53 in superimposed layers of agar. - 4 colonies.

e. fresh Y40 + Y53.

a	10^2	2.5	clear plate
b	10^2		
c	10^2		
d	4.		
e.	10^2		

Cells will react if kept in water for 24 hours + then mixed.
but not many more are found if they are kept together. ∴ recombination takes place in the agar.

Differential Centrifugation of Bacteria

Preliminary Expts.

393

ρ . Density - in sucrose buffer, centrifuge tubes washed 453 μ l.
at 10 15 mins, etc. Sediment vol.

Make	Time.	1.0	1.04	1.08	1.12	1.16	20	10
	Make	+	+	+	+	+		10
	Time							
	Make	++	++	++	++	++		1025
								70
n.g.		++	++	++	++	++		20
								50

1.16 = 20g sucrose / 100cc water

1:4 bacterial susp. in H₂O.

n-g for density.

Repeat, using 20g sucrose / 20g H₂O as $d = 1.25$. (actually 1.23)

	$\left(\frac{1 \text{ ml}}{4 \text{ ml } 1.25}\right)$	1.0	1.05	1.10	1.15	1.20	
diffuse in bottom layers, causing of junction.		++	++	+	$\pm?$	-	20m 50.
		+++	+++	\pm	+	\pm	+ 20m 50
		1.15	1.20			do.	+ 1hr.
Use heavier susp. cells.						\pm	1:30

This might achieve some separation.

Try R1
149004

Y53 + Y40 - deletion effect.

394

1/13/47.

1/2 ml of various dilutions.

	1ml + 1ml			
1.	Y53 10 ⁰	Y40. 10 ⁰	ca. 100	
2.	10 ⁻²	10 ⁰	6	
3.	10 ⁻⁴	10 ⁰	0	
4.	10 ⁰	10 ⁻²	8	
5.	10 ⁰	10 ⁻⁴	1	
6.	10 ⁻²	10 ⁻²	0	— 0.
7.	10 ⁻⁴	10 ⁻²	0	
8.	10 ⁻²	10 ⁻⁴	0	

January 15, 1947.

Inoculate Y40 in. Broz (ml/10 YB incubate 18 hours +
dilute + plate on EMB lact. 20,000 colonies examined.

3 colorless, but rather small colonies were found. Pick + test further.

1 lact + Mucoid colony was found. Pick + streak out to isolate.

all lact lact + Muc = Y69

January 17, 1947

See 383 (1-6)

P21. Colonies have taken a blue tinge. Malacostriales + conigae c Y53.

All show coloration in lytic zone c T1 virus.

5, particularly, shows few or no papillae. Y70.

1 very few papillae

Y71.

2 papillae.

3 papillae.

4 few, but some papillae

5 ~~no~~ no papillae.

6 few but some.

Y70. - Further study suggests that fewer colonies have papillae, & fewer of them are formed. Comparison should be made of some prototypic segregants. This allele may refer to Y53-lac-.

coli moffi - papillates very readily.

than 11-12, but some papillae are formed.

Attempt at transformation

January 18, 1947.

P17 - P18. 74 hour-cultures Y53 autolyse = 300 ml
^{benzene}
washed cells in NaCl under ~~these~~ 3 hours. shaken at 25°.

Sediment cells. Remove superficial film by evacuating chamber. Suspend Y40 cells in autolyse - Plate ¹⁰ ~~1~~
3 is Y40.

Control - use washed cells of above $\bar{3}$ autolysis x Y40.

See also 399.

Turbidity of autolyse was ~~more~~ ^{< 1/2} than that of the 1:100 dilution.
sup. ^{sample} ~~sample~~ overnight \bar{c} ~~heavy~~ heavy layer of benzene and repeat later.

7 fold autolyse overnight in cold.

6P19. - remove benzene from sample by evacuation.

- A. Y40 + 02-autolyse 0, also in EMF. 0
- B. Y40 + autolyse 0, 0
- C. autolyse $\bar{3}$ Y40. 0, 0.

autolyse is sterile; no prototrophs.

January 20, 1947.

A : Y40, Y10, Y64.

BM+R x LB, $\begin{matrix} +S \\ -R \end{matrix}$

-S not viable.

+R	-R	+S	-S
##		1	##-
9	7	1	11

B. 58-161, Y46, Y53

BM+S x TLB, $\begin{matrix} +R \\ -S \end{matrix}$

-R not viable.

+R	-S	+S	-R
###	###		###
###	###		###
###	###		10
###	###		###
	###-		###
	###		###
20	34	5	40

Some mistake??

See 411 for repeat

3-way cross.

BM Lac+V₁^R
Y40

Y10
TLB, Lac+V₁^S
TLB, Lac-V₁^R
Y64

→ +++
Lac+V₁^R
Lac+V₁^S
Lac-V₁^R
not Lac-V₁^S

BM Lac+V₁^S
58-161

Y46
TLB, Lac+V₁^R
TLB, Lac-V₁^S
Y53.

Lac+V₁^R
Lac+V₁^S
Lac-V₁^S
not. Lac-V₁^R

Embryos: BM Lac+V₁^R x TLB, Lac-V₁^S → all types, Lac+V₁^S rare.

BM Lac+V₁^S x TLB, Lac-V₁^R → all types, Lac+V₁^R rare.

already done!

January 18, 1947.

1/2 ml each:

1.	Y53 10 ⁰	Y40. 10 ⁰	120
2.	10 ⁻¹	10 ⁰	120
3.	10 ⁻²	10 ⁰	13
4.	10 ⁰	10 ⁻¹	60
5.	10 ⁰	10 ⁻²	8
6.	10 ⁻¹	10 ⁻¹	23
7.	10 ⁻¹	10 ⁻²	16
8.	10 ⁻²	10 ⁻¹	8
9.	10 ⁻²	10 ⁻²	1

	f(Y40)			f(Y53)		
Y53: 10 ⁰	0	120		0	120	
	-1	60	Y40:	1	120	
	-2	8	10 ⁰	2	13	
10 ⁻¹	0	120		0	60	
	-1	23	10 ⁻¹	1	23	
	-2	16		2	8	
10 ⁻²	0	13		0	8	
	-1	8	10 ⁻²	1	16	
	-2	1		2	1	

Y53+Y40	10 ⁰	120	60
	10 ⁻¹	23	23
	10 ⁻²	1	1

Mucoid segregation

400

January 17, 1947.

Y57 x Y68 (TLB, -lac - $\nabla_{1,3,5}^R$ x BM-Muc)

No prototrophs!

See 404

of 387 for mucoid seg.

Y53M

Y67 x 58-161 OK.

Y68 x Y53 OK.

58-161M

Toxicity of benzene
and removal.

401

January 19, 1947.

7P19. Layer 1/2 ml benzene on 1 ml Y40 in water. Keep on deck.
do in H_2O .

N20. Remove water layer; evacuate to remove benzene.

1. Plate to determine killing of Y40. — 0.

2. Add 1 ml fresh Y40 to fresh aqueous layer + let sit for 24h. Plate.

January 20, 1947.

P19. broz Y40, Y53 into YB + Tween ; A20 transfer likewise ; plate
 A. 1%
 B. .1%
 C. .05%

no growth effect!

in T(0) agar + 1% Tween

P19 broz Y40, Y53. into YB. etc.
 Plate into T(0) agar +

B.

A .1%	}	Tween.
B 1%		

all ca 10^2

no particular effect of Tween could be established.

January 20, 1947.

5 1 ml samples 58-161 grown 18h. in Y53. Wash + irradiate 2 mins. broi 1:100 in nut. sal.

1
2
3
4
5
1-5
survivors

58-161 is evidently more sensitive than Y53. (which has had 1 further X-ray + u.v. exposure).

~~Y64x68:~~
signations

404

January 22, 1947.

1. Y65 x 58-161 (Y10/1/7) (in 1:100 del.)
2. Y57 x Y68 (Y10 Y53/1 x BM Hue)

1. Shows no recombination prototrophs. (Is Y65 unable to recombine??)

See 379, 390

2. 1 plate is ca 100 (no sectors). (Try at See 400)

Try Y64 x Y68

BM + R x TLB₁ - S

See 385.

P21. Struck out 385-3, 4, 15.

Test 1 colony isolates on T1.

1-2	3-0	385	-R	R ¹	+ R ²
3-6	A	+S	+ S ^{5,6}	- S ^{3,4}	
7-10	B	-S	- S ^{7,8}	+ S ^{9,10}	
1	4-0	-S	- S ¹		
2-5	A	+S	+ S ^{2,3}	- S ^{4,5}	
6-9	B	-S	- S ^{6,7}	+ S ^{8,9}	
11-14	C	± S	- S ^{11,12}	+ S ^{13,14}	
15-18	D	± S	+ S ^{15,16}	- S ^{17,18}	
1-2 15-0	-R	- R ^{11,12}			
3-4 A	-S	- S ³	+ R ⁴		
5-6 B	-S	- S ⁶	+ R ⁵		
7-8 C	-S	- S ⁷	+ R ⁸		
9-10 D	-S	- S ¹⁰	+ R ⁹		

-R (B₁⁻) (Replate 3-0 also.)
 +R
 -S
 +S
 -S (B₁⁻)
 +S ?

typed -R (B₁⁻)
 -S (B₁⁻)
 and +R are present.

Test samples of above:

P: parental

clone #		Reacts	Comment		
3-1	1	-R	B ₁ ✓	B ₁ -R	
3-2	2	+R	BM TL? ✓	B ₁ -S	3 tests BML definitely
3-3	3	-S	B ₁	[B ₁ +S]	1 test BM
3-5	4	+S	B ₁ ?		
3-7	5	-S	B ₁	[++ +S]	See 408.
3-9	6	+S	++		
4-1	11	-S	B ₁ ? ✓		
4-2	12	+S	+ ? ✓		
4-6	13	-S	B ₁ ?		
15-1	21	-R	B ₁ ?	B ₁ -R	
15-3	22	-S	B ₁ ✓	B ₁ -S	
15-4	23	+R	BM ✓		
15-9	24	+R	BM ✓		
15-10	25	-S	B ₁ ✓	(B ₁ -R)(B ₁ -S).	
3-4	31	-S	B ₁		
3-6	32	+S	B ₁		
3-8	33	-S	B ₁		
3-10	34				

January 21, 1947.

250 ml eq. 24 hour cells of Y53 harvested from YB + washed.
~~with~~ autolysate 24h. under benzene at room temp.

P22. Add Y40 cells + plate.

P24 - no colonies.

January 22, 1947.

Plate "B₁⁻" colonies into T(0) agar + BMTL. Use plates which relatively few, isolated phototrophic colonies.

0 Y65 x 58-161

1 Y40 x Y53.

Test original colonies for V, R, lac - :

	"0"	"1"
1	+S	-R
2	-S	-S (+R)
3	-S (+R)	-R
4	-S	-R
5	-S	+R (-R)
6	-S	-R
7	+S	-R
8	+S	-S
9	+R	-S
10	-S	-R

Plate colonies into BMTL. Pick + test samples of colonies which arise.

	Colony	# colonies	Notes
1	+S	10 ⁵	
2	-R	1000	
3	-S; +R	1000	
4	-S	200	
5	-S	1000	
6	-S	500	
7	+S	300	
8	+S	300	
9	+R	10 ⁶	
10	-S	200	
11	-R	200	
12	-S (+R)	500	
13	-R	50	
14	-R	500	
15	+R (-R)	300	
16	-R	20	
17	-R	10 ⁶	
18	-S	200	
19	-S	500	
20	-R	200	

8 + S	}	+S = BM type -R = TLB ₁ type +R = rare type.
8 + S		
34 + S		
7 + S 7 + S	}	all +S. 1. (BM) Test for luciferase requirement.
7 + S		
8 + S	}	+R = BM type -S = TLB ₁ type is rare type [-R = B ₁ type]
10 + R		
10 + R		
2 - R		
8 + R		
9 + R		
1 - R		
10 + R		
10 + R		
9 + R		

How explain "10" - reversion of B₁⁻ ?? label must be wrong.

January 25, 1947.

Retest 405-2. in plates. Dil to ca 100/ml + pour plates \bar{c}
(475)

1. BM 346
2. BMT do
3. MBL do
4. BMTL. do.
5. BHTLB, 365